

CG-509 TARGETED PRODRUG ACTIVATION IN BREAST CANCER CELLS MEDIATED BY A RECOMBINANT ADENOVIRUS CONTAINING THE DF3 TUMOR-ASSOCIATED ANTIGEN PROMOTER WITH HERPES SIMPLEX VIRUS THYMIDINE KINASE GENE

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Patients with metastatic breast cancer usually succumb to their disease due to the non-selective nature and narrow therapeutic window of the available chemotherapeutic drugs. Recently, adenovirus-mediated transfer of the herpes simplex virus thymidine kinase (HSV-tk) gene has been shown to confer sensitivity to the cytotoxic effect of ganciclovir (GCV) in tumor cells. Since adenovirus infects a broad range of cell types, clinical application of this vector may be limited by non-specific transduction of therapeutic genes into cells other than the target cells. One strategy to circumvent this problem would be to use tumor tissue-selective/promoters or enhancers in order to direct gene expression in the desired target cell. A high molecular weight mucin-like glycoprotein, DF3 (MUC1), is a tumor-associated antigen overexpressed in 75-90% of breast cancers. We have constructed E1-deleted recombinant adenovirus containing the DF3 promoter (bps -725 to +31) with *E. coli* β -galactosidase gene (Ad.DF3- β gal) or HSV-tk gene (Ad.DF3-tk). Infection with Ad.DF3- β gal resulted in specific expression of β -galactosidase in DF3-positive breast carcinoma cell lines MCF-7, ZR-75-1 and other DF3+ tumor cell lines, while only a very low to undetectable level of expression was observed in DF3-negative tumor cell lines. Injection of Ad.DF3- β gal into human breast cancer xenografts in nude mice leads to targeted expression of the reporter gene in tumor cells but not in the surrounding normal tissue or injected skeletal muscle. MCF-7 and ZR-75-1 cells infected with Ad.DF3-tk were rendered sensitive to GCV, while Ad.DF3-tk infected DF3-negative Hs578T cells were insensitive. In a breast metastases (peritoneal carcinomatosis) model, our preliminary results demonstrate that injection of Ad.DF3-tk (1×10^9 pfu per i.p.) followed by GCV treatment resulted in partial to complete tumor regression. These results demonstrate that the recombinant adenovirus carrying the tumor-tissue selective promoter DF3 with a therapeutic gene is an efficient gene delivery system specifically targeted to DF3-positive cancer cells. These findings provide a potentially novel approach for breast cancer gene therapy.

CG-510 COMBINATION SUICIDE AND CYTOKINE GENE THERAPY FOR METASTATIC COLON CARCINOMA IN VIVO

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The efficacy and immune response of combination "suicide gene therapy" and "cytokine gene therapy" were investigated for the treatment of metastatic colon carcinoma in the liver. Tumor in the liver was generated by intrahepatic injection of a colon carcinoma cell line (MCA 26) in syngeneic BALB/c mice. After 7 days of tumor growth, recombinant adenoviral vectors containing various control and therapeutic genes were injected directly into the solid tumors, followed by treatment with ganciclovir. The tumors continued to grow in all animals treated with a control vector or a mouse interleukin 2 vector. Those animals that were treated with a Herpes Simplex Virus thymidine kinase⁺ vector, with or without the co-administration of the mouse interleukin 2 vector, exhibited dramatic tumor necrosis and regression. However, only animals treated with both vectors developed an effective systemic anti-tumoral immunity against challenges of tumorigenic doses of parental tumor cells inoculated at distant sites. The anti-tumoral immunity was associated with CD8⁺ cytolytic T-lymphocytes and were MHC II-2K^b restricted. These cytolytic T-lymphocytes were also cytolytic against a second syngeneic colon carcinoma cell line (CC36), suggesting the individual tumor cell lines shared specific tumor antigen(s). The results suggest that combination suicide and cytokine gene therapy in vivo can be a powerful approach for treatment of metastatic colon carcinoma in the liver.

CG-511 RECONSTITUTION OF Fas/APO-1 SENSITIVITY FOLLOWING ADENOVIRUS-MEDIATED

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Induction of apoptosis following monoclonal-antibody binding of the Fas/APO-1 cell-surface protein, a new member of the NGF/TNF receptor superfamily, has been described in normal and neoplastic cells. The mechanism in the α -Fas/APO-1 resistant cell that expresses the Fas/APO-1 receptor but upon antibody-binding fails to trigger apoptosis has not been identified. To determine the impact of p53 on α -Fas/APO-1 induced apoptosis, we treated an α -Fas/APO-1 resistant colon cancer cell line (KM12L4) that expresses mutant p53 with a recombinant adenovirus expressing wild-type p53, viral vector alone, or sham infection 48 h prior to treatment with α -Fas/APO-1. Fas/APO-1 expression (specific Mean Fluorescence Intensity) and apoptosis (specific DNA Fragmentation) were measured using flow cytometric analysis.

Treatment	Fas Expression (spMFI)	sp DNA Fragmentation
wt p53	6.3	66.2 %
vector	2.0	15.4 %
sham	1.4	1.4 %

Infection with wt p53 increased surface expression of Fas/APO-1 3.2-fold over vector alone group. Transfer of wt p53 similarly increased sensitivity to α -Fas/APO-1 4.3-fold over vector alone. These data demonstrate that transfer of the wt p53 gene 1) increased Fas/APO-1 expression and 2) induced α -Fas/APO-1 sensitivity. Furthermore, these data suggest a possible role of wt p53 in the apoptotic pathway triggered by binding of the Fas/APO-1 surface receptor.

CG-512 ANTISENSE DOWNREGULATION OF THYMIDYLATE SYNTHASE ACTIVITY IN HUMAN TUMOR CELLS: EFFECTS ON DRUG RESISTANCE

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Thymidylate synthase (TS) is a key enzyme in the synthesis of DNA precursors, and an important target for cancer chemotherapeutic agents such as 5-fluorouracil. Treatment with antisense nucleic acids to downregulate TS activity in cancer cells may be useful in increasing the effectiveness of such drugs. We transiently transfected human breast cancer (MCF-7) cells *in vitro* with two vectors expressing antisense TS RNA under the control of a cytomegalovirus promoter (pRC/CMV). Antisense RNAs were targeted to 30 bases of the TS mRNA including part of the stem-loop structure at the translation start site (vector 1) and 30' bases spanning the exon1/exon2 boundary (vector 2). Vectors 1 and 2 were transfected singly, or in combination. On the order of 100' copies of intact transfected vector were present in cells between 24 and 48 h following transfection, as well as low molecular weight degraded vector. Intact and degraded vector were undetectable by 5-7 days. TS enzyme activity in the MCF-7 cells (measured by an [³H]dUMP tritium release assay) was decreased by 20-80% within 2 days of transfection. In spite of successful downregulation of TS activity, subsequent resistance to the toxic effect of the antimetabolites 5-fluorouracil (in conjunction with leucovorin) and Tomudex was variable. (Supported by a grant from the LRCC Endowment Fund).